Technical Data Sheet

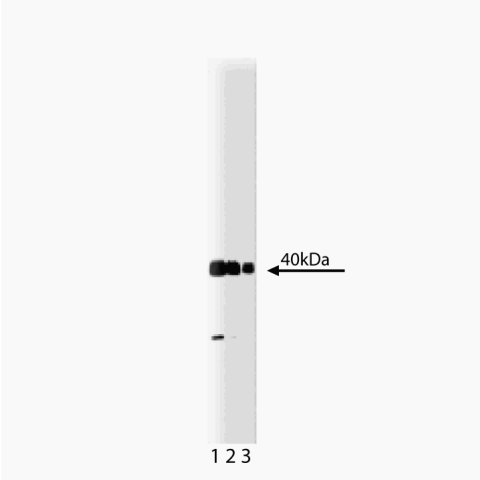
Purified Mouse Anti-Crk

Product Information

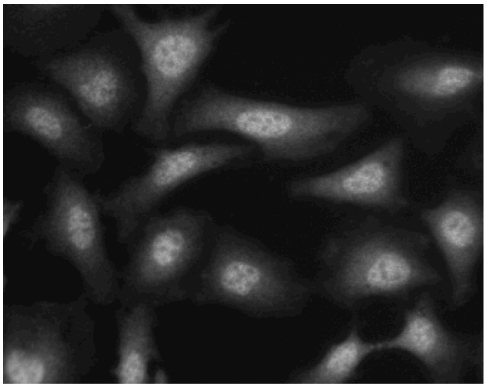
Material Number:	**610036**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	22/Crk
Immunogen:	Human Crk aa. 102-304
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in Development: Bovine, Chicken, Dog, Frog, Mouse, Rat
Target MW:	40 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Crk was first isolated as the v-*crk* oncogene from chicken retroviruses CT10 and ASV-1. All human cell lines examined to date express a 40 kDa Crk protein. In addition, there is variable expression of 42 kDa and 28 kDa Crk proteins. The c-*crk* gene is one of a class of genes, such as *Nck* and *GRB2/ASH*, which encode proteins that consist mainly of SH2 and SH3 domains. These proteins function as adaptor molecules in tyrosine kinase signal transduction pathways. The SH2 domains interact with phosphotyrosine-containing peptides, while the SH3 domains can enhance this interaction and/or bind to other cellular components. Both the SH2 and SH3 domains of the human Crk protein are required for differentiation of PC12 cells. Thus, Crk has a role in an NGF-induced signaling pathway that involves activation of p21ras. Furthermore, three proteins of 118 kDa, 125 kDa, and 136 kDa which specifically bind to the Crk SH3 domain have been identified.



Western blot analysis of Crk on a HeLa lysate. *Lane 1: 1:5000, lane 2: 1:10000, lane 3: 1:20000 dilution of the Crk antibody.*



Immunofluorescent staining of HeLa (ATCC CCL-2) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the Triton™ X-100 perm protocol and the anti-Crk antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a BD Pathway 855 Bioimager system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X/100 and alcohol perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

BD Biosciences

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Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611449	HeLa Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Cho SY, Klemke RL. Purification of pseudopodia from polarized cells reveals redistribution and activation of Rac through assembly of a CAS/Crk scaffold. *J Cell Biol.* 2002; 156(4):725-736. (Clone-specific: Immunoprecipitation, Western blot)

Fournier TM, Lamorte L, Maroun CR, et al. Cbl-transforming variants trigger a cascade of molecular alterations that lead to epithelial mesenchymal conversion. *Mol Biol Cell.* 2000; 11(10):3397-3410. (Clone-specific: Immunoprecipitation)

Girardin SE, Yaniv M. A direct interaction between JNK1 and CrkII is critical for Rac1-induced JNK activation. *EMBO J.* 2001; 20(13):3437-3446. (Clone-specific: Immunofluorescence, Immunoprecipitation, Western blot)

Liu J, Kimura A, Baumann CA, Saltiel AR. APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. *Mol Cell Biol.* 2002; 22(11):3599-3609. (Clone-specific: Immunoprecipitation, Western blot)

Miller CT, Chen G, Gharib TG, et al. Increased C-CRK proto-oncogene expression is associated with an aggressive phenotype in lung adenocarcinomas. *Oncogene.* 2003; 22(39):7950-7957. (Clone-specific)

Smith JJ, Evans EK, Murakami M, et al. Wee1-regulated apoptosis mediated by the crk adaptor protein in Xenopus egg extracts. *J Cell Biol.* 2000; 151(7):1391-1400. (Clone-specific: Immunoprecipitation, Western blot)

Smith JJ, Richardson DA, Kopf J, Yoshida M, Hollingsworth RE, Kornbluth S. Apoptotic regulation by the Crk adapter protein mediated by interactions with Wee1 and Crm1/exportin. *Mol Cell Biol.* 2002; 22(5):1412-1423. (Clone-specific)